Clinical Nutrition ESPEN 39 (2020) 46–52

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Randomized Controlled Trial

The clinical effects of a carbohydrate-reduced high-protein diet on glycaemic variability in metformin-treated patients with type 2 diabetes mellitus: A randomised controlled study☆

Mads N. Thomsen [a, *], Mads J. Skytte [a], Arne Astrup [b], Carolyn F. Deacon [c, d], Jens J. Holst [c, d], Sten Madsbad [e], Thure Krarup [a], Steen B. Haugaard [a, f], Amirsalar Samkani [a]

[a] Dept. of Endocrinology, Copenhagen University Hospital Bispebjerg, Copenhagen, Denmark
[b] Dept. of Nutrition, Exercise and Sports, University of Copenhagen, Denmark
[c] Endocrinology Research Section, Dept. of Biomedical Sciences, University of Copenhagen, Denmark
[d] Section for Translational Physiology, NNF Center for Basic Metabolic Research, University of Copenhagen, Denmark
[e] Dept. of Endocrinology, Copenhagen University Hospital Amager Hvidovre, Copenhagen, Denmark
[f] Dept. of Internal Medicine, Copenhagen University Hospital Amager Hvidovre, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 17 February 2020
Accepted 3 July 2020

Keywords:
Type 2 diabetes
Low-carbohydrate diet
Glycaemic variability
Continuous glucose monitoring

SUMMARY

Background & aims: High glycaemic variability (GV) is associated with late complications in type 2 diabetes (T2D). We hypothesised that a carbohydrate-reduced high-protein (CRHP) diet would reduce GV acutely in patients with T2D compared with a conventional diabetes (CD) diet.

Methods: In this controlled, randomised crossover study, 16 patients with metformin-treated T2D (median (IQR) age: 64.0 (58.8–68.0) years; HbA_{1c}: 47 (43–57) mmol/mol; duration of T2D: 5.5 (2.8–10.3) years) were assigned to an energy-matched CRHP diet and CD diet (31E%/54E% carbohydrate, 29E%/16E% protein and 40E%/30E% fat, respectively) for two separate 48-h intervention periods. Interstitial continuous glucose monitoring (CGM) was performed to assess accepted measures of glycaemic variability, i.e. standard deviation (SD) around the sensor glucose level; coefficient of variation in percent (CV); mean amplitude of glucose excursions (MAGE); continuous overlapping net glycaemic action ($CONGA_1$, $CONGA_4$) of observations 1 and 4 h apart; and mean absolute glucose (MAG) change.

Results: All indices of glycaemic variability (mean ± SD) were significantly reduced during CRHP diet compared with CD diet; including SD (1.0 ± 0.3 (CRHP) vs 1.6 ± 0.5 mmol/L (CD)), CV (12.3 ± 3.8 vs 19.3 ± 5.5%), MAGE (2.3 ± 0.9 vs 4.2 ± 1.3 mmol/L), $CONGA_1$ (0.8 ± 0.3 vs 1.5 ± 0.4 mmol/L), $CONGA_4$ (1.4 ± 0.5 vs 2.5 ± 0.8 mmol/L), and MAG change (0.9 ± 0.3 vs 1.4 ± 0.4 mmol/L/h) ($p < 0.001$ for all). Compared with the CD diet, the CRHP diet improved the diurnal glucose profile by reducing 24-h mean sensor glucose (7.7 ± 1.6 vs 8.6 ± 2.0 mmol/L).

Conclusions: In T2D patients treated with diet and metformin, two days of iso-energetic replacement of dietary carbohydrates by protein and fat reduced all indices of glycaemic variability by 36%–45% when compared with a conventional diabetes diet. These data may support reduction of carbohydrates as dietary advice for T2D patients.

Clinicaltrials.gov identifier: NCT02472951.

☆ Results previously presented at: EASD 2018. 54th Annual Meeting of the European Association for the Study of Diabetes. 1–5 October 2018 in Berlin, Germany. Poster presentation. DES 2019, Annual Meeting of the Danish Endocrinology Society, 18–19 January 2019 in Nyborg, Denmark. Oral presentation.

* Corresponding author. Copenhagen University Hospital Bispebjerg, Bispebjerg Bakke 23, 2400, Copenhagen, NV, Denmark.
E-mail address: mads.norvin.thomsen@regionh.dk (M.N. Thomsen).

https://doi.org/10.1016/j.clnesp.2020.07.002
2405-4577/

1. Introduction

The UK Prospective Diabetes Study (UKPDS) documented the importance of chronic sustained hyperglycaemia for development of late diabetic complications and mortality in type 2 diabetes (T2D) patients [1]. Since, levels of glycosylated haemoglobin (HbA_{1c}) are considered the best indicator of glycaemic control and reductions in HbA_{1c} are regarded a key factor in reducing risk of diabetes-related complications [2]. Excessive rise of postprandial glucose (PPG) is a frequent manifestation of dysglycaemia in T2D [3] and a major contributor to overall hyperglycaemia in T2D patients [4]. In addition, PPG fluctuations have been demonstrated to be independently correlated with T2D-associated morbidity and mortality [5,6]. A growing body of evidence suggests that the size, frequency and duration of these fluctuations, i.e. the glycaemic variability (GV), irrespective of the magnitude of hyperglycaemia, may confer additional risks for the development of micro-and macrovascular diabetic complications [7], although well-designed randomised studies illustrating the association are missing [8,9].

Nutrition therapy plays an integral role in overall diabetes management, but studies examining the ideal diet for T2D patients are inconclusive and evidence-based guidelines are warranted [10]. Nevertheless, growing evidence supports that diets reduced in carbohydrates enhance glycaemic control [11,12], in agreement with the finding that the dietary carbohydrate content is one of the greatest contributors to postprandial hyperglycaemia [13], and proteins have been shown to be a viable substitute for carbohydrate in improving glycaemic control [14]. However, in the published studies, assessment of glycaemic control has been limited to measurements of HbA_{1c} and fasting glucose [11], which do not capture the diurnal glucose variations. With the availability of continuous glucose monitoring (CGM) systems, information about this can now be obtained, and emerging evidence supports its use in improving [15] and complementing HbA_{1c} measurements as a marker of glycaemic control [16,17]. CGM measurements are usually analysed electronically to provide various indices of GV. Currently, in the absence of a GV 'gold standard', an appropriate alternative approach is to consider a range of GV indices that provide measures of different aspects of intra- and inter-day glucose fluctuations [18]. Studies systematically evaluating the effects of carbohydrate reduction on such indices are, so far, scarce.

The aim of this study was to investigate, in subjects with T2D, whether two days of dietary carbohydrate reduction with iso-energetic substitution with protein and fat would improve the diurnal glucose profile, evaluated both with standard measures of glycaemia and with the most often used indices of GV, compared to a conventionally recommended diet.

2. Materials and methods

2.1. Study population

The study included 16 non-smoking patients diagnosed with T2D and treated with metformin (Table 1). Weight was maintained throughout the study. No patients dropped out. Patients were recruited from August 2015 to June 2016 via the medical records at Bispebjerg University Hospital, Copenhagen, Denmark. Fifty-one patients were contacted, 18 were screened (Supplementary Fig. 1) and 16 met the inclusion criteria: men and post-menopausal women with T2D and below the age of 70 years. Patients with liver or kidney disease or critical illness were excluded; the eligibility criteria has been described in more details elsewhere [19]. Diagnosis of T2D was based on the criteria of the American Diabetes Association [20] and absence of plasma glutamate decarboxylase (GAD-65) antibodies. Patients provided written, informed consent to the study protocol which was approved by both the Health Ethics Committee of Copenhagen and the Danish Data Protection Agency. The study was registered at clinicaltrials.gov (ID: NCT02472951).

Table 1
Baseline characteristics.

Characteristics	
Male sex (n) (%)	14 (88)
Age (years)	64.0 (58.8–68.0)
Weight (kg)	94.0 ± 17.3
Height (cm)	176.2 ± 7.0
Body mass index (kg/m^2)	30.1 ± 4.4
Daily total energy expenditure (KJ)	10298 ± 1356
Glycosylated haemoglobin (mmol/mol)	47 (43–57)
Duration of type 2 diabetes (years)	5.5 (2.8–10.3)

Data are expressed as mean ± SD or median (25–75 percentile) unless otherwise specified.

2.2. Design and intervention

In this controlled, open-label, randomised crossover study, patients underwent two 48-h intervention periods separated by a 2–8 week washout period. Patients were randomised by drawing of blinded ballots to start with either a carbohydrate-reduced high-protein (CRHP) diet or a conventional diabetes (CD) diet (according to Nordic Nutrition Recommendations [21]). Following an overnight fast, the assigned breakfast and lunch meals were ingested at the Endocrine Research Unit of the hospital. Dinner and pre- and post-dinner snacks were provided for home consumption. This was repeated the following day. Prior to each 48-h intervention, a control dinner was provided to be consumed at home the evening before. During the intervention period, patients were sedentary in a reclined position at the research unit and were furthermore asked to abstain from physical exercise when at home. Alcohol and strenuous physical activity were prohibited for three days prior to experimental days. No tea, coffee or calorie-containing beverages or foods other than provisioned were allowed during the experimental days. Macronutrient compositions of the CRHP/CD diet for every meal were 31E%/54E% carbohydrate, 29E%/16E% protein and 40E%/30E% total fat; a detailed description has been given elsewhere [19] (Supplementary Table 1). The diets were comparable with respect to added sugar, glycaemic index and fatty acid composition. The intervention diets were iso-energetic and corresponded to each patient's estimated daily total energy expenditure (TEE), calculated by multiplying resting energy expenditure (RES) with a physical activity level of 1.4. RES was derived from measures of fat-free mass and fat mass, obtained from Dual-Energy X-ray Absorptiometry (DXA) (Lunar iDXA, GE Healthcare, Madison, WI, USA) [19]. Thirty percent of TEE was ingested at each main meal (breakfast, lunch and dinner) and the remaining 10% as pre- and post-dinner snacks. All meals were weighed out and prepared by trained kitchen staff instructed in the protocol at Bispebjerg University Hospital kitchen.

2.3. CGM procedure

Diurnal glucose profiles were obtained using the iPro2 CGM system and the Enlite glucose sensor (Medtronic MiniMed, Inc., Northridge, CA), which provided abdominal subcutaneous interstitial glucose level readings at 5-min intervals over 48 h, corresponding to a total of 576 measurements. Previous work has shown this to be a reliable [22] and accurate method, when matched against YSI-2300 (Yellow Springs Instruments, Inc., Yellow Springs,

Ohio, USA) [23]. The CGM sensor was mounted at least 16 h prior to each 48-h intervention period. Capillary blood glucose was measured with the Contour Next Link (Medtronic MiniMed, Inc., Northridge, CA), previously validated, hand-held glucose meter [24], at least 5 times per day for calibration of the CGM measurements. Stored CGM data were exported to online software (CareLink iPro; Medtronic), together with the capillary measurements, to convert measured signals into glucose values, as per the manufacturer's instruction. Accuracy of glucose values was evaluated according to number of valid calibrations and the mean absolute difference percentage (MAD%). All glucose profiles were included in the analysis.

2.4. Outcomes

CGM measurements were analysed for each 48-h intervention period (Fig. 1). Glycaemic control was assessed as following: mean sensor glucose (MSG) level; postprandial glucose (PPG), mean of glucose readings over 4 h post-breakfast and -lunch; postprandial glucose excursions (PPGE), i.e. magnitude of the postprandial peak post-breakfast and -lunch; maximum and minimum sensor glucose; area under the curve (AUC) above a level of 7.8 and 10 mmol/L. AUC was calculated using the trapezoidal rule. The percentage of time spent in range (3.9–10 mmol/L) or hyperglycaemia (>10 mmol/L), as defined by the American Diabetes Association glycaemic control targets [25], were assessed, as well as the time spent above a level of 7.8 mmol/L (as research shows this glucose level seldomly to be exceeded by healthy people with normal glucose tolerance [26]). The intra-day GV indices included standard deviation (SD) around the sensor glucose level; coefficient of variation in percent (CV) (SD/MSG x 100); mean amplitude of glucose excursions (MAGE); continuous overlapping net glycaemic action ($CONGA_n$) of observations n hours apart; mean absolute glucose (MAG) change. MAGE, as introduced by Service et al. [27], is the arithmetic mean of glucose excursions (either upward or downward, decided by the direction of first excursion) exceeding 1.0 SD of the mean glucose for a given day. In the present study, MAGE was computed using an automated algorithm of the original method [28,29], which has been found to correlate strongly with the original method ($r = 0.95$, $p < 0.001$) and to result in similar results between the two methods of calculation at all MAGE values [29]. $CONGA_n$ is calculated as the SD of differences between glucose values at each 5 min time point and the one n hours previously [30]. MAG change is the absolute increments and decrements of glucose from peaks to nadirs per hour, and has, as a GV index, been found applicable to CGM data [31]. The inter-day variability was assessed using mean of daily differences (MODD), defined as the arithmetic mean of differences between glucose values obtained at the same time point on two consecutive days [32]. In the analysis of group differences between diet interventions, each parameter, with the exception of MODD, was calculated for each day and included as the mean of both days on each intervention.

2.5. Data analysis and statistics

The reported measures of glycaemia and GV were secondary analyses of explorative endpoints according to state-of-the-art in the literature concerning clinical significance and applicability of CGM data. Data were examined for normality visually and with Shapiro–Wilk test, after which group differences between diet interventions were compared using paired sample t-tests or Wilcoxon signed-rank tests where appropriate. Additionally, analyses of correlation were performed using Pearson or Spearman correlation where appropriate. Data are presented as mean ± SD or median (25–75 percentile) values unless otherwise specified. A minimal detectable difference of GV of 22% given a standard deviation of 30% between interventions was calculated to be detected with $n = 16$ participants, with power 80% and a significance level of 0.05, the level of p-values used on all parameters to reject the null hypotheses. All statistical analyses were performed using R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

3. Results

3.1. Glycaemia

Compared with the CD diet, the CRHP diet was found to reduce (mean ± SD) 24-h MSG (7.7 ± 1.6 vs 8.6 ± 2.0 mmol/L) and PPG levels (8.5 ± 1.9 vs 10.1 ± 2.4 mmol/L and 8.0 ± 1.5 vs 9.4 ± 2.3 mmol/L, after breakfast and lunch, respectively), $p < 0.001$ for all (Table 2). The PPG excursions were reduced on the CRHP compared with the CD diet, with excursions differing approximately three- (1.3 ± 0.9 vs 3.4 ± 1.6 mmol/L) and two-fold (1.3 ± 0.8 vs 2.8 ± 1.3 mmol/L) after breakfast and lunch, respectively, $p < 0.001$ for both. Total 24-h maximum glucose values were reduced while consuming the CRHP

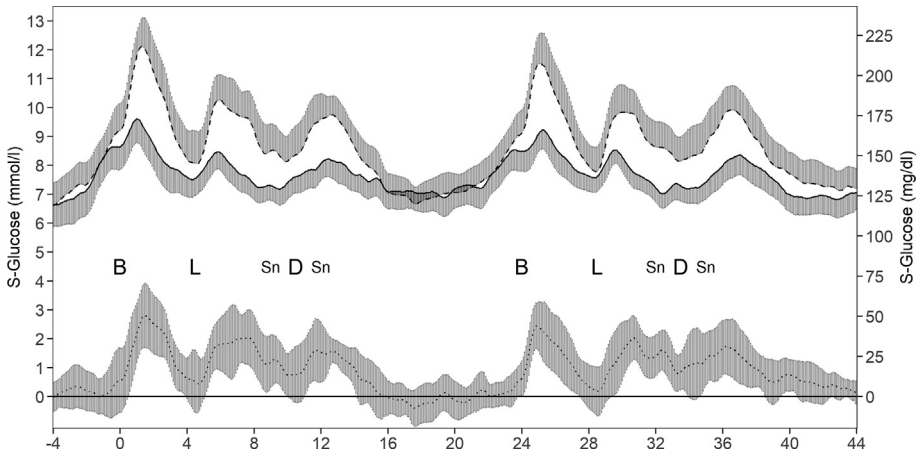

Fig. 1. Glucose profiles of 48-h continuous glucose monitoring. The three lines represent CD diet (dashed, only upper limit of 95% CI displayed), CRHP diet (solid, only lower limit of 95% CI displayed) and the difference between CRHP and CD diet (dotted, with 95% CI). B, breakfast (t_0, t_{24}); L, lunch ($t_{4.5}$, $t_{28.5}$); Sn, snack; D, dinner; postprandial phase after breakfast, t_0-t_4, t_{24}-t_{28} and postprandial phase after lunch, $t_{4.5}$-$t_{8.5}$, $t_{28.5}$-$t_{32.5}$.

Table 2
Indices of glycaemia and glycaemic variability by continuous glucose monitoring.

n = 16	Diet[a]		Difference[b]	Reduction[c] (%)	p-value
	CD	CRHP			
Glycaemic Parameters					
MSG (mmol/L)	8.6 ± 2.0	7.7 ± 1.6	0.9 (0.5–1.3)	10.4	<0.001[d]
PPG (mmol/L)					
Breakfast	10.1 ± 2.4	8.5 ± 1.9	1.6 (1.0–2.2)	15.8	<0.001[d]
Lunch	9.4 ± 2.3	8.0 ± 1.5	1.4 (0.8–2.0)	14.9	<0.001[d]
PPGE (mmol/L)					
Breakfast	3.4 ± 1.6	1.3 ± 0.9	2.1 (1.5–2.8)	62.7	<0.001[d]
Lunch	2.8 ± 1.3	1.3 ± 0.8	1.6 (0.8–2.3)	55.1	<0.001[d]
Maximum (mmol/L)	12.6 ± 2.5	10.1 ± 2.0	2.5 (1.9–3.2)	20.2	<0.001[d]
Minimum (mmol/L)	5.3 (4.6–6.5)	5.7 (4.8–6.7)	−0.1 (−0.9 to 0.3)		0.20[e]
AUC (h x mmol/L)					
>10.0 mmol/L	6.4 (0.6–12.3)	0.1 (0.0–0.9)	6.1 (0.5–11.9)		0.002[e]
>7.8 mmol/L	25.4 (5.9–47.2)	6.5 (1.9–12.4)	17.5 (5.3–25.7)		<0.001[e]
Time Spent in					
Range (3.9–10.0 mmol)	80.4 (62.2–96.7)	97.2 (94.5–100)	−13.6 (−24.1 to −1.6)		0.003[e]
Hyperglycaemia					
>10.0 mmol/L	18.0 (3.1–37.8)	0.8 (0.0–5.5)	14.0 (0.8–24.1)		0.003[e]
>7.8 mmol/L	51.3 (25.8–91.7)	37.7 (11.4–56.5)	17.4 (6.1–23.3)		0.001[e]
Glycaemic Variability					
SD (mmol/L)	1.6 ± 0.5	1.0 ± 0.3	0.7 (0.5–0.9)	43.0	<0.001[d]
CV (%)	19.3 ± 5.5	12.3 ± 3.8	7.0 (5.2–8.8)	36.4	<0.001[d]
MAGE (mmol/L)	4.2 ± 1.3	2.3 ± 0.9	1.9 (1.4–2.4)	44.6	<0.001[d]
$CONGA_1$ (mmol/L)	1.5 ± 0.4	0.8 ± 0.3	0.6 (0.5–0.8)	43.8	<0.001[d]
$CONGA_2$ (mmol/L)	2.1 ± 0.7	1.2 ± 0.4	0.9 (0.7–1.2)	44.4	<0.001[d]
$CONGA_4$ mmol/L)	2.5 ± 0.8	1.4 ± 0.5	1.1 (0.8–1.3)	43.3	<0.001[d]
MAG change (mmol/L/h)	1.4 ± 0.4	0.9 ± 0.3	0.6 (0.4–0.7)	39.3	<0.001[d]
MODD (mmol/L)	0.9 (0.7–1.1)	0.6 (0.5–0.8)	0.2 (0.1–0.6)		0.002[e]

CD, conventional diabetes; CRHP, carbohydrate-reduced high-protein; MSG, mean sensor glucose; PPG, postprandial glucose; PPGE, postprandial glucose excursion; AUC, area under the curve; SD, standard deviation; CV, coefficient of variation; MAGE, mean amplitude of glucose excursions; $CONGA_n$, continuous overlapping net glycaemic actions of n hours apart; MAG change, mean absolute glucose change; MODD, mean of daily differences.
[a] Mean ± SD or median (25–75 percentile).
[b] mean (95% CI) or median (25–75 percentile).
[c] Percentage of difference divided by CD.
[d] Tested by paired sample t-test.
[e] Tested by Wilcoxon signed-rank test.

compared with the CD diet (10.1 ± 2.0 vs 12.6 ± 2.5 mmol/L, $p < 0.001$), whereas no significant difference of 24-h minimum glucose levels (median (IQR)) was observed (5.7 (4.8–6.7) vs 5.3 (4.6–6.5) mmol/L, $p = 0.20$). AUCs in the hyperglycaemic range (>10 mmol/L) were reduced (0.1 (0.0–0.9) vs 6.4 (0.6–12.3) h x mmol/L, $p = 0.002$) as were those above 7.8 mmol/L (6.5 (1.9–12.4) vs 25.4 (5.9–47.2) h x mmol/L, $p < 0.001$) by the CRHP compared with CD diet. Patients consuming the CRHP diet were more likely to spend time in range (3.9–10.0 mmol/L) than those consuming the CD diet (97.2 (94.5–100) vs 80.4 (62.2–96.7)%, $p = 0.003$), and correspondingly less time in the hyperglycaemic range (0.8 (0.0–5.5) vs 18.0 (3.1–37.8)%, $p = 0.003$) and above 7.8 mmol/L (37.7 (11.4–56.5) vs 51.3 (25.8–91.7)%, $p = 0.001$), CRHP vs CD diet, respectively.

3.2. Glycaemic variability

Intra-day indices of GV were all reduced (mean ± SD) ($p < 0.001$ for all) on the CRHP compared with the CD diet (Table 2), including SD (1.0 ± 0.3 vs 1.6 ± 0.5 mmol/L), CV (12.3 ± 3.8 vs 19.3 ± 5.5%), MAGE (2.3 ± 0.9 vs 4.2 ± 1.3 mmol/L), $CONGA_1$ (0.8 ± 0.3 vs 1.5 ± 0.4 mmol/L), $CONGA_2$ (1.2 ± 0.4 vs 2.1 ± 0.7 mmol/L), $CONGA_4$ (1.4 ± 0.5 vs 2.5 ± 0.8 mmol/L) and MAG change (0.9 ± 0.3 vs 1.4 ± 0.4 mmol/L/h). In addition, all indices were uniformly reduced for all 16 patients while consuming the CRHP diet (Fig. 2). Compared with the CD diet, the CRHP diet reduced inter-day variability, measured as MODD (median (IQR)) (0.6 (0.5–0.8) vs 0.9 (0.7–1.1) mmol/L, $p = 0.002$). The indices of intra-day GV (disregarding assigned diet) were shown to correlate strongly with each other (Pearson's $r = 0.84–0.98$, $p < 0.001$ for all) and with MODD (Spearman's $\rho = 0.69–0.83$) (Supplementary Table 2).

4. Discussion

In the present study, there was an acute improvement in the diurnal glucose profiles in subjects with T2D when changed from the conventional diabetes diet to an iso-energetic carbohydrate-reduced diet with corresponding increase in proteins and fats. Diurnal glucose profiles were estimated by the commonly used measures of glycaemia and indices of both intra- and inter-day variability. As no single measure yet covers all aspects of dynamic glucose profiles, a multifaceted approach is needed to analyse continuous glucose measurements.

If maintained, the improvement in mean glucose of 0.9 mmol/L with the CRHP diet could lead to a theoretical reduction in HbA_{1c} of ~6 mmol/mol [33]. Postprandial glucose excursions have been shown to strongly predict HbA_{1c} levels in well-controlled T2D patients [4] and a reduction to contribute independently to reduce the risk of CVD [34]. The CRHP diet reduced the glucose AUC and the time spent in hyperglycaemia (>10 mmol/L) and above 7.8 mmol/L, which is of relevance, as guidelines regarding glycaemic control in T2D advocate blood glucose below 10 mmol/L [25]. Individuals with normal glucose tolerance rarely exceed blood glucose levels of 7.8 mmol/L after a meal, but even well-controlled T2D patients do [35,36].

The SD index during consumption of the CD diet was similar to that reported by Kohnert et al. [36] in a cross-sectional study of a

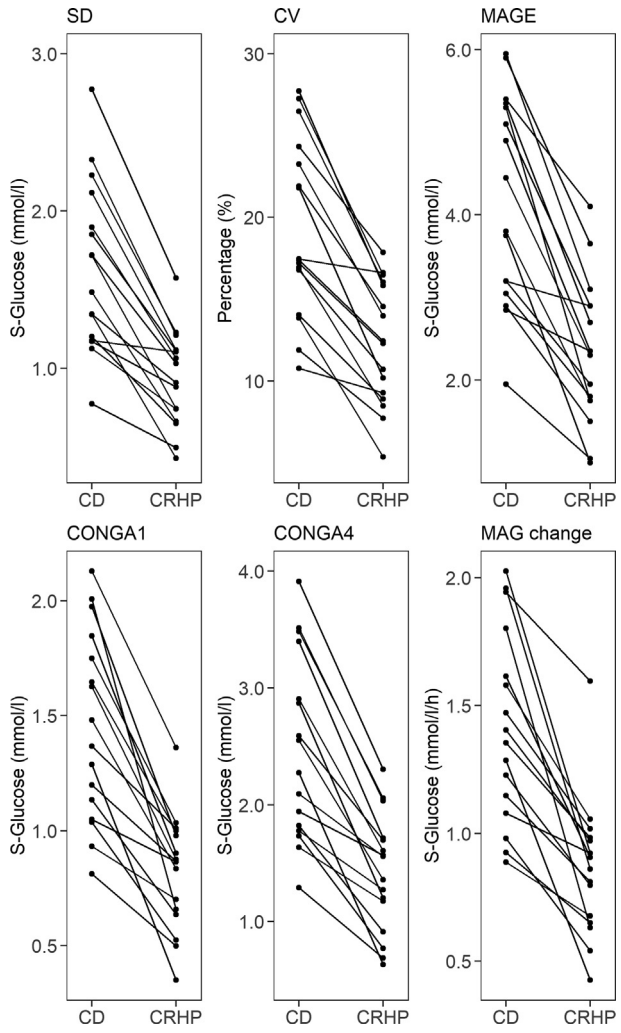

Fig. 2. Individual intra-day GV differences of the 16 participants. SD, standard deviation; CV, coefficient of variation; MAGE, mean amplitude of glucose excursions; CONGA, continuous overlapping net glycaemic action; MAG change, mean absolute glucose change.

comparable population with well-controlled T2D (HbA_{1c}<7%), while SD index was reduced during the CRHP diet. Mori et al. [37] compared the acute effect of a standard carbohydrate (55-60E%) with a low-carbohydrate (32E%) liquid diet, both with a protein content of ~17E%, in tube fed T2D patients under severe caloric restriction reducing SD 1.1 mmol/L (36.5%) on the low compared with standard carbohydrate liquid diet. Reducing carbohydrate content while maintaining protein content seems, under these conditions, to have a major impact on SD. In another study by Tay et al. [38] comparing a very low-carbohydrate (LC, 14E%) with a standard carbohydrate diet (HC, 53E%) in moderately well-controlled T2D patients, absolute values of SD after 24 weeks of intervention were 1.1 and 1.5 mmol/L, respectively; a difference seemingly persisting medium-term [39].

As SD is easy to calculate and well-validated, it is commonly reported, and is a preferred method for quantifying GV from CGM data [18,40]. A limitation is that glucose profile data rarely follow a Gaussian distribution, which is required for the use of SD [41]. Despite this shortcoming, SD remains a fairly robust measure of GV, as a linear relation between interquartile range and SD has been established [42], and numerous studies have shown very strong correlations between SD and commonly used GV measurements [18]. In the present study (Supplementary Table 2), and in accordance with the literature [43], SD was found to correlate with the mean of glucose readings (Pearson's $r = 0.46$). However, if the SD is corrected for the mean glucose value, the so-called CV index is obtained which has been proposed as a primary measure of variability [44]. In the present study, the CRHP diet resulted in an absolute reduction of CV by 7.0%, a relative decrease of 36.4%. Importantly, both diets resulted in stable glucose levels classified as CV<36% [44], which in part could be explained by the participants only being treated with metformin.

The magnitude of estimates in the present study for MAGE while consuming the CD diet was similar to that found in a study by Kohnert et al. [36]. The study conducted by Mori et al. [37] showed an absolute reduction of 2.5 mmol/L (32.5% relative reduction) of MAGE between diets in favour of the low-carbohydrate diet, while the absolute values of MAGE found by Tay et al. [38] (LC 2.9 and HC 3.9 mmol/L) differed by 1.0 mmol/L. For quantification of glucose oscillations, MAGE has proven applicable to analyse intermittent and continuous glycaemic data [28] and has been proposed as the 'gold standard' metric for measuring GV [45]. Service et al. [27] proposed the MAGE index to only include excursions larger than 1.0 SD of the diurnal glucose measurements to disregard minor excursions present in healthy individuals. The MAGE index has been criticised due to operator dependency [18] and ambiguity regarding the arbitrary definitions of peaks and nadirs [42]. As the original method was found to be an unreliable measure of GV [22], an automated algorithm applicable to interstitial CGM systems has been recommended [28,46].

All three indices of $CONGA_n$ were reduced comparably during CRHP diet, regardless of the duration of time frame (n hours), with almost identical (43.3–44.4%) relative reductions in our study. The absolute values of $CONGA_n$ on either diet increased with increasing n, which is a tendency described previously by Rodbard [18], with absolute differences amounting to 0.6, 0.9 and 1.1 mmol/L, respectively, for $CONGA_1$, $CONGA_2$ and $CONGA_4$. Tay et al. [38] found a carbohydrate-reduced diet to have comparable influence on $CONGA_1$ and $CONGA_4$ as found in the present study, which persisted after two years of follow-up [39]. McDonnell et al. [30] introduced the $CONGA_n$ as a mathematically consistent and objective expression of GV from continuous glucose tracings while taking all variability into account. However, this index has been criticized due to the failure in discriminating pathological excursions from excursions that might be considered as noise [41].

MAG change on both diet interventions in our study was smaller than previously reported [31], particularly on the CRHP diet. The importance of MAG change in T2D is largely unknown, although recently, an important point regarding its use in T2D was made. In a reanalysis of the HEART2D study, MAG change decreased significantly, whereas reductions of SD and MAGE were not statistically significant [40]. MAG change was calculated from seven-point glucose profiles, correlating with CGM measurements [31]. Notably, MAG change reflects kinetics of glycaemic change per unit of time rather than representing a true assessment of the magnitude of glucose excursions [31,47].

We show here that inter-day GV is very much affected by the intervention diet, with MODD values comparable to and below those seen in healthy individuals while consuming the CD and CRHP diet, respectively [30]. MODD has previously been reported to be much higher in patients with T2D [48]. MODD is, however, influenced by differences in meal timing and physical activity, making comparisons between studies with different designs difficult [40]. MODD results should, therefore, be interpreted with caution and generally require detailed information about lifestyle [30]. Improving MODD may be useful, as MODD has been associated with oxidative stress and diabetic neuropathy, independently of HbA_{1c}, in patients with T2D [49,50].

A strength of the current study is the standardization of intervention periods, as patients remained sedentary and ingested most of the assigned daily meals under supervision at breakfast and lunch, though dietary compliance at home could not be verified. Due to the small day-to-day variability, each parameter could be computed reliably for both consecutive study days on each diet. Furthermore, the analysis included a complete set of data from all patients and comprised of state-of-the-art clinically relevant markers of glycaemia and variability derived from CGM data. To minimize inaccuracies resulting from the CGM measurements [41], frequent calibrations were performed in accordance with the manufacturer's instructions. CGM systems have proven unreliable at extreme values (outside 2.2–22.2 mmol/L) [23], but this was of little concern in the present study population of well-controlled T2D patients on metformin monotherapy. The size and homogeneity of the population in the present study (the majority being male with good glycaemic control) limits the generalizability of the present findings to larger, more heterogeneous populations with T2D.

In conclusion, moderately reduced intake of carbohydrates with concomitant iso-energetic increased intake of protein and fat acutely improved glycaemic control and reduced GV in T2D patients treated with diet and metformin. The improvements were evident in all subjects for all indices of intra-day GV and also inter-day GV was normalized.

Author contributions

The authors' contributions were as follows: M. J. S., A. A., C. F. D., J. J. H., S. M., T. K., S. B. H. and A. S. designed and conducted the research; M. N. T. and A. S. performed the data analysis and wrote the manuscript; M. J. S., A. A., C. F. D., J. J. H., S. M., T. K. and S. B. H. coedited the manuscript; M. N. T. was primary responsible for the manuscript and its final contents; all authors read and approved the final manuscript.

Declaration of Competing Interest

No conflicts or competing financial interests are declared.

Acknowledgements

Conducting this study was made possible because of a grant from the Danish Dairy Research Foundation and the support of the participants and the help of the kitchen staff at Copenhagen University Hospital Bispebjerg. The design, analysis or writing of this article was not influenced by The Danish Dairy Research Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2020.07.002.

References

[1] UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998 Sep 12;352(9131):837–53.
[2] Selvin E, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powe NR, et al. Meta-analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. Ann Intern Med 2004 Sep 21;141(6):421–31.
[3] Bonora E, Corrao G, Bagnardi V, Ceriello A, Comaschi M, Montanari P, et al. Prevalence and correlates of post-prandial hyperglycaemia in a large sample of patients with type 2 diabetes mellitus. Diabetologia 2006 May;49(5):846–54.
[4] Monnier L, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). Diabetes Care 2003 Mar;26(3):881–5.
[5] Cavalot F, Petrelli A, Traversa M, Bonomo K, Fiora E, Conti M, et al. Postprandial blood glucose is a stronger predictor of cardiovascular events than fasting blood glucose in type 2 diabetes mellitus, particularly in women: lessons from the San Luigi Gonzaga Diabetes Study. J Clin Endocrinol Metab 2006 Mar;91(3):813–9.
[6] Hanefeld M, Fischer S, Julius U, Schulze J, Schwanebeck U, Schmechel H, et al. Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. Diabetologia 1996 Dec;39(12):1577–83.
[7] Tay J, Thompson CH, Brinkworth GD. Glycemic variability: assessing glycemia differently and the implications for dietary management of diabetes. Annu Rev Nutr 2015;35:389–424.
[8] Nalysnyk L, Hernandez-Medina M, Krishnarajah G. Glycaemic variability and complications in patients with diabetes mellitus: evidence from a systematic review of the literature. Diabetes Obes Metab 2010 Apr;12(4):288–98.
[9] Madsbad S. Impact of postprandial glucose control on diabetes-related complications: how is the evidence evolving? J Diabetes Complications 2016 Mar;30(2):374–85.
[10] American Diabetes A. 4. Lifestyle management: standards of medical care in diabetes-2018. Diabetes care 2018 Jan;41(Suppl 1):S38–50.
[11] Ajala O, English P, Pinkney J. Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. Am J Clin Nutr 2013 Mar;97(3):505–16.
[12] Huntriss R, Campbell M, Bedwell C. The interpretation and effect of a low-carbohydrate diet in the management of type 2 diabetes: a systematic review and meta-analysis of randomised controlled trials. Eur J Clin Nutr 2018 Mar;72(3):311–25.
[13] Nuttall FQ, Almokayyad RM, Gannon MC. Comparison of a carbohydrate-free diet vs. fasting on plasma glucose, insulin and glucagon in type 2 diabetes. Metabolism 2015 Feb;64(2):253–62.
[14] Nuttall FQ, Schweim K, Hoover H, Gannon MC. Effect of the LoBAG30 diet on blood glucose control in people with type 2 diabetes. Br J Nutr 2008 Mar;99(3):511–9.
[15] American Diabetes A. 7. Diabetes technology: standards of medical care in diabetes-2020. Diabetes care 2020 Jan;43(Suppl 1):S77–88.
[16] Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical targets for continuous glucose monitoring data interpretation: Recommendations from the international consensus on time in range. Diabetes Care 2019 Aug;42(8):1593–603.
[17] Lu J, Ma X, Zhou J, Zhang L, Mo Y, Ying L, et al. Association of time in range, as assessed by continuous glucose monitoring, with diabetic retinopathy in type 2 diabetes. Diabetes Care 2018 Nov;41(11):2370–6.
[18] Rodbard D. Interpretation of continuous glucose monitoring data: glycemic variability and quality of glycemic control. Diabetes Technol Therapeut 2009 Jun;11(Suppl 1):S55–67.
[19] Samkani A, Skytte MJ, Kandel D, Kjaer S, Astrup A, Deacon CF, et al. A carbohydrate-reduced high-protein diet acutely decreases postprandial and diurnal glucose excursions in type 2 diabetes patients. Br J Nutr 2018 Apr;119(8):910–7.
[20] American Diabetes A. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. Diabetes Care 2018 Jan;41(Suppl 1):S13–27.
[21] Nordic Nutrition Recommendations 2012. Nordic Nutrition Recommendations NNR 2012 - intergrating nutrition and physical activity. Nord 2014;2012:2004. 002.
[22] Terada T, Loehr S, Guigard E, McCargar LJ, Bell GJ, Senior P, et al. Test-retest reliability of a continuous glucose monitoring system in individuals with type 2 diabetes. Diabetes Technol Therapeut 2014 Aug;16(8):491–8.
[23] Bailey TS, Ahmann A, Brazg R, Christiansen M, Garg S, Watkins E, et al. Accuracy and acceptability of the 6-day Enlite continuous subcutaneous glucose sensor. Diabetes Technol Ther 2014 May;16(5):277–83.
[24] Bernstein R, Parkes JL, Goldy A, Brown D, Harrison B, Chu A, et al. A new test strip technology platform for self-monitoring of blood glucose. J Diabetes Sci Technol 2013 Sep 1;7(5):1386–99.
[25] American Diabetes A. 6. Glycemic targets: standards of medical care in diabetes-2018. Diabetes care 2018 Jan;41(Suppl 1):S55–64.
[26] International Diabetes Federation Guideline Development. Guideline for management of postmeal glucose in diabetes. Diabetes Res Clin Pract 2014 Feb;103(2):256–68.
[27] Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, Taylor WF. Mean amplitude of glycemic excursions, a measure of diabetic instability. Diabetes 1970 Sep;19(9):644–55.
[28] Baghurst PA. Calculating the mean amplitude of glycemic excursion from continuous glucose monitoring data: an automated algorithm. Diabetes Technol Ther 2011 Mar;13(3):296–302.
[29] Fritzsche G, Kohnert KD, Heinke P, Vogt L, Salzsieder E. The use of a computer program to calculate the mean amplitude of glycemic excursions. Diabetes Technol Ther 2011 Mar;13(3):319–25.
[30] McDonnell CM, Donath SM, Vidmar SI, Werther GA, Cameron FJ. A novel approach to continuous glucose analysis utilizing glycemic variation. Diabetes Technol Ther 2005 Apr;7(2):253–63.
[31] Kohnert KD, Heinke P, Fritzsche G, Vogt L, Augstein P, Salzsieder E. Evaluation of the mean absolute glucose change as a measure of glycemic variability using continuous glucose monitoring data. Diabetes Technol Ther 2013 Jun;15(6):448–54.

[32] Molnar GD, Taylor WF, Ho MM. Day-to-day variation of continuously monitored glycaemia: a further measure of diabetic instability. Diabetologia 1972 Nov;8(5):342–8.
[33] Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, et al. Translating the A1C assay into estimated average glucose values. Diabetes Care 2008 Aug;31(8):1473–8.
[34] Cavalot F. Do data in the literature indicate that glycaemic variability is a clinical problem? Glycaemic variability and vascular complications of diabetes. Diabetes Obes Metab 2013 Sep;15(Suppl 2):3–8.
[35] Ceriello A. The glucose triad and its role in comprehensive glycaemic control: current status, future management. Int J Clin Pract 2010 Nov;64(12):1705–11.
[36] Kohnert KD, Augstein P, Heinke P, Zander E, Peterson K, Freyse EJ, et al. Chronic hyperglycemia but not glucose variability determines HbA1c levels in well-controlled patients with type 2 diabetes. Diabetes Res Clin Pract 2007 Sep;77(3):420–6.
[37] Mori Y, Ohta T, Shiozaki M, Yokoyama J, Utsunomiya K. The effect of a low-carbohydrate/high-monounsaturated fatty acid liquid diet and an isoleucine-containing liquid diet on 24-h glycemic variability in diabetes patients on tube feeding: a comparison by continuous glucose monitoring. Diabetes Technol Ther 2012 Jul;14(7):619–23.
[38] Tay J, Luscombe-Marsh ND, Thompson CH, Noakes M, Buckley JD, Wittert GA, et al. A very low-carbohydrate, low-saturated fat diet for type 2 diabetes management: a randomized trial. Diabetes Care 2014 Nov;37(11):2909–18.
[39] Tay J, Thompson CH, Luscombe-Marsh ND, Wycherley TP, Noakes M, Buckley JD, et al. Effects of an energy-restricted low-carbohydrate, high unsaturated fat/low saturated fat diet versus a high-carbohydrate, low-fat diet in type 2 diabetes: a 2-year randomized clinical trial. Diabetes Obes Metab 2018 Apr;20(4):858–71.
[40] Siegelaar SE, Holleman F, Hoekstra JB, DeVries JH. Glucose variability; does it matter? Endocr Rev 2010 Apr;31(2):171–82.
[41] Service FJ. Glucose variability. Diabetes 2013 May;62(5):1398–404.
[42] DeVries JH. Glucose variability: where it is important and how to measure it. Diabetes 2013 May;62(5):1405–8.
[43] Rodbard D. Clinical interpretation of indices of quality of glycemic control and glycemic variability. Postgrad Med 2011 Jul;123(4):107–18.
[44] Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International consensus on use of continuous glucose monitoring. Diabetes Care 2017 Dec;40(12):1631–40.
[45] Monnier L, Colette C, Boegner C, Pham TC, Lapinski H, Boniface H. Continuous glucose monitoring in patients with type 2 diabetes: why? When? Whom? Diabetes Metab 2007 Sep;33(4):247–52.
[46] Cameron FJ, Donath SM, Baghurst PA. Measuring glycaemic variation. Curr Diabetes Rev 2010 Jan;6(1):17–26.
[47] Monnier L, Colette C. Glycemic variability: can we bridge the divide between controversies? Diabetes Care 2011 Apr;34(4):1058–9.
[48] Zhou J, Jia W, Bao Y, Ma X, Lu W, Li H, et al. Glycemic variability and its responses to intensive insulin treatment in newly diagnosed type 2 diabetes. Med Sci Monit 2008 Nov;14(11):CR552–8.
[49] Xu F, Zhao LH, Su JB, Chen T, Wang XQ, Chen JF, et al. The relationship between glycemic variability and diabetic peripheral neuropathy in type 2 diabetes with well-controlled HbA1c. Diabetol Metab Syndrome 2014;6(1):139.
[50] Ohara M, Fukui T, Ouchi M, Watanabe K, Suzuki T, Yamamoto S, et al. Relationship between daily and day-to-day glycemic variability and increased oxidative stress in type 2 diabetes. Diabetes Res Clin Pract 2016 Dec;122:62–70.